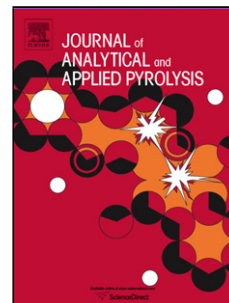


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# Upgraded production of (1*R*,5*S*)-1-hydroxy-3,6-dioxabicyclo[3.2.1]octan-2-one from cellulose catalytic pyrolysis and its detection in bio-oils by spectroscopic methods

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## ABSTRACT

Catalytic pyrolysis of cellulose was carried out focusing on the selective production of the anhydrosugar (1*R*,5*S*)-1-hydroxy-3,6-dioxabicyclo[3.2.1]octan-2-one (LAC), a promising chiral chemical for application in organic synthesis. The catalyst Sn-MCM-41, montmorillonite K10 or aluminum titanate nanopowder was used by a suitable pyrolysis reactor performing the processes at 500 °C and 350 °C. After a workup adapted to optimize the production of LAC and to facilitate the following purification, its amount in the produced bio-oil samples was established by <sup>1</sup>HNMR spectroscopy using the standard-addition method. A further quantitative analysis was based on FT-IR technique performed using a CaF<sub>2</sub> liquid cell and employing the calibration-curve method. Both the methods, which do not require any pre-treatment steps, provided comparable values (±1 %) in terms of LAC abundance in bio-oil samples and validation of the FT-IR based method made it a rapid and efficient tool for quantitative LAC detection also without need of carbonyl band deconvolution. The data showed that *i*) Sn-MCM-41 promoted the highest LAC production by pyrolysis at 500 °C (7.6±0.1 wt.% from cellulose), with a lower than 1% decrease in the presence of this catalyst after a regeneration cycle, *ii*) the cheap and eco-friendly montmorillonite K10 emerged as the best alternative, with a yield from cellulose of 4.8±0.1 wt.% at 500 °C and 4.6±0.1 wt.% at 350 °C.

**Keywords:** Cellulose, Bio-oil, Catalytic pyrolysis, Anhydrosugar, Quantitative NMR, FT-IR cell analysis.

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## 1.Introduction

Catalytic pyrolysis of cellulose is an extensively investigated process for the production of biofuels and chemicals [1]. Among the most commonly obtained anhydro-monosaccharide products, some of them (Fig. 1) can have a practical relevance as chiral building blocks in the production of added-value fine chemicals [2]. Recently, a particular interest has been focused on the hydroxylactone LAC [3], used in the synthesis of a polyester by co-polymerization with L-lactide [4], for obtaining anew tetrahydrofuran amino acid structurally isosteric to the dipeptide alanine-glycine [5] and as a promising precursor for the synthesis of enantiomerically pure compounds with potential application in drug discovery. It justifies the efforts made and currently attempt in the selective production of LAC enriched bio-oil, of which the best reported results are given by using aluminium titanate (AlTi) nanopowders [3], tin-containing silica molecular sieves (Sn-MCM-41) [6] and the eco-friendly and cheap montmorillonite K10 (MK10) [7] as solid catalysts.

[Insert here Fig.1]

The most common qualitative and quantitative analyses for chemical characterization of pyrolysis bio-oils are based on the combined gas chromatography-mass spectrometry (GC-MS) and gas chromatography-flame ionization detector (GC-FID) techniques, although GC analysis can only detect about 40 wt.% of pyrolysis bio-oil components. Otherwise, due to its feature to analyze also not volatile components, liquid chromatography (LC) is applied under different conditions depending on the bio-oil fractions of interest. High-resolution mass spectrometry (HRMS) has been also adopted, although different ionization methods could be necessary also for a single class of compounds depending from their polarity [8].

Until the last decade, spectroscopic methods including nuclear magnetic resonance (NMR) and Fourier transform-infrared spectroscopy (FT-IR) have found applications mainly restricted to kinetic studies on pyrolysis mechanism and bio-oil aging, rather than for composition analysis of bio-oils themselves [9,10]. The recent increased number of employs is due to their manageability and ability in characterizing almost each bio-oil component without the need for sample pre-purification.

In the FT-IR analysis, mid-infrared ( $3300\div600\text{ cm}^{-1}$ ) is the most considered region of the spectrum since it allows to reveal most of the molecular functional groups present in pyrolysis blends such acids, esters, ketones, aldehydes, ethers, and alcohols [11]. Due to the *difficulty in performing quantitative analysis with FT-IR methods, many reports have focused on the evaluation of absorption band intensity by comparing different spectra and approximately evaluate the composition change of a blend* [12]. Nevertheless analytical methods based on the FT-IR quantification have been reported for pyrolysis carbonyl products using liquid cells [11] and light gasses where thermogravimetric (TG)-FTIR resulted a reliable method for quantitative measurements of thermal decomposition species from biomass pyrolysis [13].

NMR spectroscopy shows advantages in the investigation of bio-oils, due to its ability to detect all the components dissolved in a suitable deuterated solvent, and to give a quantitative information by signal integration with the assistance of a reference compound. A series of applications were reported on the employment of quantitative  $^1\text{H}$ NMR and  $^{13}\text{C}$ NMR analysis, for the evaluation of anhydrosugars [14] and carbonyl compounds [15].

This study aims to upgrade the production of the hydroxylactone LAC *via* catalytic pyrolysis of cellulose under different catalytic conditions and temperature values by using a detailed apparatus and an optimized workup procedure. Its evaluation in bio-oils has been established by a quantitative NMR method, based on the standard-addition technique, used also for validating a faster approach by FT-IR technique in solution.

## 2. Experimental

### 2.1. Materials

Microcrystalline cellulose was purchased from Merck KGaA. Levoglucosenone (= (1S,5R)-6,8-dioxabicyclo[3.2.1]oct-2-en-4-one, LGO), 5-(hydroxymethyl)-2-furaldehyde (HMF), 2-furaldehyde (furfural, FF) and montmorillonite K10 (Sigma Aldrich), titanium alluminate powder (Alfa Aesar) were commercially available and Sn-MCM-41 was synthesized according to a reported method [6]. All catalysts were activated in muffle at 550°C for six hours before their use. Regeneration of Sn-MCM-41 was carried out by calcination of the exhaust catalyst in a muffle at 550 °C for six hours. Isopropanol of LC-MS-Chromasolv grade used for bio-oil solutions to be analyzed by FT-IR was purchased from Fluka.

### 2.2. Off-line pyrolysis apparatus and procedure for bio-oil production

Catalytic pyrolysis reaction was performed using a home-made pyrolysis reactor. It consists in a quartz phial fixed on a stainless steel flange in which two stainless steel pipes (internal diameter 4 mm) connect the reactor, toward the flange, respectively to the nitrogen line and to the condensation apparatus (Fig. 2). A porcelain combustion boat containing cellulose (200 mg) and the catalyst (200 mg of Sn-MCM-41 or MK10, or 66 mg of AlTi [3]) were inserted into the reactor connected to a tube furnace (Carbolite CTF 22/65/550). The temperature was checked within the reaction volume by a thermocouple thermometer and the nitrogen flow was regulated by a Thorpe tube-flowmeter (25 cm<sup>3</sup>min<sup>-1</sup>). The condensation apparatus was carried out by a bubbler containing acetonitrile (20 mL) cooled at -35 °C by a liquid nitrogen acetone bath. Catalytic pyrolysis was carried out at 350 °C and 500 °C for 5 minutes reaction time. The solvent and the volatile components of bio-oil were removed by using a rotary evaporator (water bath at 60°C and vacuum at 30 mmHg). This light fraction was analyzed for the crude bio-oil from Sn-MCM-41 catalyzed pyrolysis at 500 °C. It did not contain LAC whereas water was present (as established by <sup>1</sup>HNMR analysis in CDCl<sub>3</sub> and IR spectrum) and was evaluated as 12 wt.% of the LAC enriched bio-oils; the light and heavy fractions of bio-oil corresponded to a yield of 3.1 wt.% and 28.1 wt.% from cellulose, respectively. The LAC enriched bio-oils were immediately analyzed by the suitable spectroscopic techniques and subjected to work up for LAC purification.

[Insert here Fig. 2]

### 2.3. HPLC purification of LAC

LAC was purified by preparative HPLC technique using a Hitachi Merck L-6200 apparatus equipped with a 250x10 mm column packed with Merck-Lichrosorb CN (7µm) and eluting with hexane/isopropanol 9:1 with a 5 mLmin<sup>-1</sup> flow, under UV detection (Jasco UV-975) at λ = 224 nm. Evaporation of solvent from the fraction collected at retention time of 16.7 min gave LAC, the

118 purity of which was confirmed by comparison of  $^1\text{H}$ NMR spectrum in  $\text{CDCl}_3$  with reported data  
119 [3].

#### 120 2.4. NMR Analysis of bio-oils

121  $^1\text{H}$ NMR spectra were recorded on a Bruker Avance 400 spectrometer by using a 5 mm BBI  
122 probe, at 400 MHz in  $\text{CDCl}_3$ ,  $\delta$  values are in ppm relative to the solvent residual signal at 7.25 ppm,  
123 with an 8 s acquisition time averaged on 32 transients. Samples for  $^1\text{H}$ NMR analysis were prepared  
124 by solubilizing the filtrated bio-oils (1.2 mg) in  $\text{CDCl}_3$  (0.6 mL) previously treated with basic  
125 alumina to avoid acidic traces. Four following rates of HPLC purified LAC (10  $\mu\text{L}$  of a 58.7 mM  
126 solution in  $\text{CDCl}_3$ ) were added as the standard for determining its amount in each bio-oil. After  
127 each addition, the quantitative evaluation was based on the ratio between the peak area of the LAC  
128 signal at 4.295 ppm (dd,  $J=11.8$ , 1.7 Hz, 1H, H-4ax [3], integrated in the range 4.272÷4.322 ppm),  
129 and the peak area of the signal at 6.12 ppm (d,  $J=10.0$  Hz, H-3, integrated in the range 6.102÷6.143  
130 ppm), previously assigned to the anhydrosugar LGO thanks to an available standard. The areas  
131 evaluations were performed on baseline-corrected spectra, repeating the measurements five times  
132 and taking into account the average values. The calibration curve was reported with peak areas as a  
133 function of LAC molar concentration in bio-oils. The quantification of LAC was performed for bio-  
134 oil samples obtained by 500 °C pyrolysis processes in the presence of Sn-MCM-41, regenerated Sn-  
135 MCM-41, montmorillonite K10 and titanium alluminate nanopowder.

#### 136 2.5. FT-IR Analysis of bio-oils

137 FT-IR spectra were recorded using a Bruker Tensor 27/37 spectrometer with spectral  
138 resolution of 4  $\text{cm}^{-1}$  at room temperature. Each bio-oil sample was analyzed as an isopropanol  
139 solution at a 25  $\text{mg mL}^{-1}$  concentration, loaded in a  $\text{CaF}_2$  cell (0.21 mm path length). The  
140 corresponding spectrum, averaged on 16 scans, was baseline-corrected by the Opus-Bruker  
141 software. The calibration curve for LAC evaluation was obtained by recording eight spectra of  
142 isopropanol solutions containing known concentrations of pure LAC in the range 2÷100 mM. The  
143 resulting data were plotted as an intensity/molar concentration graph.

#### 144 2.6. DFT calculated IR spectrum of LAC

145 Starting from LAC structure generated by molecular mechanics software PC Model 7.0 [16],  
146 quantum chemical calculations were performed on a Pentium Quad Core PC 2.8 GHz with 8 GB  
147 RAM using the Gaussian 03 W revision E.01 package program set.[17] Restricted Density  
148 Functional Theory (DFT) calculations were applied for geometry optimization in methanol using  
149 the polarizable continuum model (PCM) and invoking gradient geometry optimization. The basis  
150 set of choice was 6-31G(d,p) for all the atoms. The gradient-corrected DFT with the functional  
151 B1B95 [18] was used for the calculation. The optimized structural parameters were employed in the  
152 vibrational energy calculations at the DFT levels to characterize all stationary points as minima.  
153 Then, vibrationally averaged nuclear positions were adopted for harmonic vibrational energy  
154 calculations, resulting in IR wavenumbers together with intensities and force constants. For the  
155 optimized structure, no imaginary wavenumber modes were obtained, proving that a local minimum  
156 on the potential energy surface was actually found. The Gaussian checkpoint files were converted  
157 using the freq check utility in the Gaussian program. The converted files were then used to view the

animated vibrational motions of the molecule in Gauss View 3.0. For the theoretical IR spectrum, a Lorentzian line shape with a line width of  $4.0\text{ cm}^{-1}$  was used. The computed wavenumbers were scaled by factor 0.955, which is suggested for B1B95/6-31G(d) calculations [19].

### 3. Results and discussion

#### 3.1. Production of LAC enriched bio-oils

Our aim was to obtain bio-oils enriched in LAC which could make easier and more efficient its recovery with a grade of purity suitable for applications in organic synthesis. It was previously observed that the weakness in its production in less than one gram scale lies in the liquid chromatographic purification, especially working with bio-oils where it was present in a low amount. In particular silica gel resulted a harmful stationary phase for its acidity, able to downgrade also pure LAC, whereas cyano (CN) normal phase allowed a major recovery. Otherwise, reversed stationary phase was avoided due to the reactivity of water as a component in mobile phase, because able to hydrolyze the lactone group.

Cellulose was subjected to pyrolysis in the presence of three catalysts which were previously reported giving the highest production of LAC: AlTi nanopowders [3], Sn-MCM-41 [6] and MK10 [7], at  $500\text{ }^{\circ}\text{C}$  and  $350\text{ }^{\circ}\text{C}$ . The corresponding LAC enriched bio-oils were obtained from the original acetonitrile solutions by further filtrations and evaporation (Experimental) able to remove the most volatile compounds. The yield of the obtained residual bio-oils resulted to be deeply affected by the operating conditions, mainly heating-up speed, residence time in the reactor volume and efficiency in cooling of condensable products. At  $350\text{ }^{\circ}\text{C}$  the reaction afforded mostly char with only  $8\div 15\text{ wt.}\%$  yield of bio-oil, whereas at  $500\text{ }^{\circ}\text{C}$  it could be obtained up to  $20\div 25\text{ wt.}\%$  yield, in accordance with reported data [20]. In addition to the temperature of the condensation apparatus, the nitrogen flow rate in the reactor volume proved to be a crucial factor for obtaining pyrolysis oil: a nitrogen flow rate of  $25\text{ cm}^3\text{min}^{-1}$ , measured by a Thorpe flowmeter, and a condenser cooled at  $-35\text{ }^{\circ}\text{C}$  demonstrated to best afford the bio-oil [21]. Our yields, resulting much lower than those commonly reported [3,6,7], could be related to the pyrolysis apparatus. In the ten centimeters stainless steel tube connecting the reactor with the condensation trap, the temperature rapidly decreased to  $200\text{ }^{\circ}\text{C}$  causing the premature condensation of the highest boiling point compounds. In detail LG (b.p.  $384\text{ }^{\circ}\text{C}/760\text{ mmHg}$ ) is not present in LAC enriched bio-oils, whereas the LAC absence was confirmed in the condensed phases by NMR analysis. A further explanation for the obtained crude bio-oil yields can come from the total absence of water, as discussed below.

#### 3.2. Quantitative NMR analysis of LAC in bio-oils

$^1\text{H}$ NMR spectra for each LAC-enriched bio-oil were recorded in  $\text{CDCl}_3$  solution at a dilution able to dissolve all the components at a compatible concentration with NMR detection limit. The spectra mostly differ for the intensity of signals assigned to LAC by comparison with a pure sample and only weakly for the presence of different compounds (Fig. 3). According to the standard-addition method [22], two sufficiently isolated and in intensity comparable signals were chosen in order to establish the peak area ratio. The best choice turned out to be the doublet at  $4.295\text{ ppm}$  related to the axial H-4 in LAC [3] and the doublet at  $6.121\text{ ppm}$  for the reference compound, corresponding to H-3 in levoglucosenone (LGO, Fig.1). The spectra were then recorded for all the investigated bio-oils obtained by pyrolysis at  $500\text{ }^{\circ}\text{C}$ , after a series of additions of standard LAC

199 solution. The resulting data allowed to draw a graph reporting the relative peak areas *versus* molar  
200 LAC concentration (Fig. 4).

201 [Insert here Fig. 3]

202 [Insert here Fig. 4]

203 The equation of the obtained curve was established by least square linear regression and the  
204 relative LAC concentration in each bio-oil sample was obtained by graphic extrapolation (Table 1).  
205 The data indicated that the LAC richest bio-oil came from Sn-MCM-41 catalyzed pyrolysis (27.6  
206 wt.%), and that this catalytic efficiency slightly decreases (lower than 1%) after one regenerative  
207 step. Montmorillonite K10 and aluminum-titanate catalysts provided a lower abundance of LAC  
208 (17.8 wt.% and 10.9 wt.%, respectively) associated to a higher complexity of the bio-oil  
209 composition. It was reported that for a common quantitative NMR method, accuracy approaches  
210 2%, a value which was observed only in the data for both Sn-MCM-41 and recycled Sn-MCM-41  
211 catalysts. It is due to the parameters affecting the accuracy, like the complexities of the blends and  
212 of the spectral regions where signals for both the analyte and reference compound are taken into  
213 account [22].

214 [Insert here Table 1]

### 215 3.3. Quantitative FT-IR analysis of LAC in bio-oils

216 Based on a recent report on FT-IR quantitative method for carbonyl compounds in bio-oils  
217 [11], a new procedure was planned to quantify the hydroxylactone LAC in bio-oils. The procedure  
218 included the acquisition of IR spectra for bio-oil solution in isopropanol, by using a  $\text{CaF}_2$  liquid  
219 cell, which affords an overall analysis window between  $2850\text{ cm}^{-1}$  and  $1470\text{ cm}^{-1}$ . This range is  
220 ideal for the detection of carbonyl compounds and carboxylic acid derivatives ( $1870\div1570\text{ cm}^{-1}$ ),  
221 enclosing LAC molecule. The LAC enriched bio-oil obtained in our treatment could contain  
222 selective carbonyl compounds according to their melting points. Levoglucosenone (LGO), 5-  
223 (hydroxymethyl)-2-furaldehyde (HMF) and 2-furaldehyde (furfural, FF) are the most predictable by  
224 literature [3, 6, 7], as confirmed by experimental NMR data. In Fig.5 their minimal contribution to  
225 LAC absorption is shown.

226 [Insert here Fig. 5]

227 The choice of isopropanol as the solvent is based on its advantages, including a high enough  
228 boiling point and the ability of dissolving very viscous samples [11]. A preliminary analysis on a  
229 pure LAC solution in isopropanol showed a strong absorption band at  $1747\text{ cm}^{-1}$  attributable to the  
230  $\text{C}=\text{O}$  stretching, which resulted very similar to the value obtained in methanol solution at similar  
231 concentration ( $1747\text{ cm}^{-1}$ ). For this latter solvent, DFT calculation using the polarizable continuum  
232 model was possible, providing the theoretical IR spectrum of LAC which allowed to assign the  
233 vibration at  $1749\text{ cm}^{-1}$  to the lactone group. These data suggest that the absorption wavenumber  
234 value is not affected by the kind of alcohol solvent, as expected for the involvement of similar  
235 intermolecular interactions.

236 A calibration curve was obtained acquiring the FT-IR spectra for LAC isopropanol solutions  
237 at different concentrations and plotting the absorption/molar concentration graph (Fig. 6).

FT-IR spectra recorded for isopropanol solutions of LAC enriched bio-oil samples, obtained from pyrolysis carried out by changing both the catalyst and temperature, present a complex profile in the window of interest (Fig. 7). However the LAC absorption band at  $1747\text{ cm}^{-1}$  is quite clearly detectable allowing a direct evaluation of its intensity. The obtained calibration curve slope enabled to determine the LAC concentration in each enriched bio-oil sample.

[insert here Fig. 6]

[insert here Fig. 7]

The results adequately agree with the ones given by the quantitative NMR analysis, with a smooth overrating in FT-IR method on average 1 wt.% LAC abundance. This agreement also indicated a neglectable contribution to LAC absorption by the presence of carbonyl products.

Additional quantification of LAC from cellulose pyrolysis at  $350\text{ }^{\circ}\text{C}$  was also evaluated (Table 2). The obtained results confirmed the best efficiency of Sn-MCM-41 catalyst also after recycling, although with a small decrease at  $350^{\circ}\text{C}$ , while MK10 and AlTi nanopowders proved to better work at lower temperature. The comparison of the data acquired by NMR and FT-IR quantitative analyses showed values within a 1% deviation for LAC quantification in the bio-oil samples under investigation. In addition this result indicated that there are no significant contributions of carbonyl products to the IR absorption of LAC.

By this validation of FT-IR method, it resulted as a fast and efficient tool able to follow also bio-oil degradation in the course of time, being its storage a discussed question [23]. In detail we used the FT-IR method for monitoring LAC degradation, the abundance of which was observed to rapidly decrease at room temperature in any solvent, until disappearing after about 20 days for each investigated bio-oil sample. This decrement proved to proceed more slowly at  $-20\text{ }^{\circ}\text{C}$  in different organic solvents, with an evaluated  $10\div 12\text{ wt.}\%$  decrease in 30 days. It was established that the best condition to store bio-oil samples in order to preserve LAC abundance, was in dichloromethane solution at  $-80\text{ }^{\circ}\text{C}$ .

[Insert here Table 2]

### 3.4. Evaluation of water presence in bio-oils

It was reported that the absorption of water in the IR region, typical of oxygen-containing functional groups, must be taken into account. In particular the presence of the broad band at  $1650\text{ cm}^{-1}$ , due to its scissoring vibration, can positively contribute to the intensity of other peaks [24]. Therefore this contribution has been evaluated for the different bio-oil samples under investigation. In Fig. 8 the IR absorptions for the isopropanol solution of the LAC-enriched bio-oil, from pyrolysis catalyzed by recycled Sn-MCM-41, has been correlated to both the absorptions of solutions in the same solvent of both pure LAC and water in different concentrations. It was evident that water contribution to the intensity of the band assigned to C=O lactone group in LAC structure is negligible. In addition it makes the FT-IR method adaptable also for bio-oil containing water up to 40 wt.%. A more tightening evaluation came from  $^1\text{H}$ NMR analysis, where we did not see the water typical broad signal at 1.59 ppm that was not present in the LAC-enriched bio-oils spectra. It was so possible to establish the absence of water, attributable to the adopted work up. Otherwise, water is commonly present in a generic crude bio-oil because normally produced



through dehydration reaction happening during pyrolysis, up to 25 wt.% as confirmed just by <sup>1</sup>HNMR spectroscopy in comparison with Karl Fischer titration [25].

[Insert here Fig. 8]

### 3.5. Comparative production of LAC from cellulose

By the evaluation of LAC yields from pyrolysis using different catalysts and temperature, the data reported in Table 3 point out to : *i*) a 7.6 wt.% in the presence of 1:1 mass ratio of Sn-MCM-41/ cellulose at 500 °C, to be compared with a 5.2÷5.9 wt.% yield reported at the same temperature by Torri et al.[6], which *ii*) slightly lowers by using the recycled catalyst; *iii*) a 7.3 wt.% in the presence of 1:1 mass ratio of MK10/ cellulose at 500 °C, to be compared with a 1.6÷2.7 wt.% yield obtained at the 450 °C by Rutkowski [7], and *iv*) a 2.4 wt.% by treating AlTi nanopowders/ cellulose in 1:3 mass ratio at 350 °C, to be compared with the value of 6.0 wt.% reported by Fabbri at the same temperature [3]. In every way, the isolation of pure LAC resulted an easier and more efficient procedure by using the configuration reactor and the workup for the LAC enriched bio-oils here reported. The procedure implying the use of Sn-MCM-41 at 500°C emerged as the method producing the highest yields of LAC from cellulose, but the method of choice appears the one based on MK10, which is industrially available in large amounts at a low cost, eco-friendly, and regenerable. In this case the yield of LAC is acceptable, moreover the obtained bio-oils do not contain water and are produced in all the catalytic cellulose pyrolysis here reported in reduced amounts, making easier the purification of LAC. In detail, the bio-oils were produced from cellulose with yields in the range 23÷27 wt.% at 500 °C and 15÷18 wt.% at 350 °C, in line with a known reduced bio-oil amount from lower temperature processes [1].

[Insert here Table 3]

## 4. Conclusions

With the aim of obtaining pure LAC by an efficient procedure for its application in organic synthesis, an upgraded method for the production of this hydroxylactone has been selected carrying out a cellulose pyrolysis at 500 °C and 350 °C, in the presence of different catalyst (Sn-MCM-41 before and after regeneration, montmorillonite K10 and AlTi nanopowder) by using a suitable reactor and bio-oil workup.

The obtained LAC enriched bio-oils were analyzed in CDCl<sub>3</sub> solutions by a quantitative <sup>1</sup>HNMR method using the standard-addition of pure LAC. This was used to validate a quantitative FT-IR procedure for isopropanol solutions of bio-oils, based on the intensity of C=O lactone absorption band, which were not affected by the contribution of water presence. It proved to be a less time consuming and wieldier procedure, able to quantify LAC amount in bio-oils without the need to evaluate the areas by a deconvolution process. Based on the amounts of LAC in bio-oil samples evaluated by these spectroscopic methods and its corresponding production from cellulose, Sn-MCM-41 resulted the most efficient catalyst even after a regenerative process, although montmorillonite K10 emerged as the system of choice, due to its reported advantages like environmental compatibility, low cost, and ability to be regenerated.

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## 324 References

- 325 [1] A. V. Bridgwater, Review of fast pyrolysis of biomass and product upgrading, *Biomass and Bioenergy* 38 (2012)  
 326 68-94.
- 327 [2] X. Zhu, Q. Lu, Production of Chemicals from Selective Fast Pyrolysis of Biomass, In: M. N. B. Momba (Eds),  
 328 Biomass, Publisher online Sciyo, 2010, pp. 147-164.
- 329 [3] D. Fabbri, C. Torri, I. Mancini, Pyrolysis of cellulose catalysed by nanopowder metal oxides: production and  
 330 characterisation of a chiral hydroxylactone and its role as building block, *Green Chem.* 9 (2007) 1374-1379.
- 331 [4] P. Dobrzynski, D. Fabbri, C. Torri, J. Kaczmarczyk, M. Pastusiak, A novel hydroxy functionalized polyester  
 332 obtained by ring opening copolymerization of L-lactide with a pyrolysis product of cellulose, *J. Polym. Sci. Part A:*  
 333 *Polym. Chem.* 47 (2009) 247-257.
- 334 [5] A. Defant, I. Mancini, D. Fabbri, An efficient route towards a new branched tetrahydrofuran  $\delta$ -sugar amino acid  
 335 from a pyrolysis product of cellulose, *Amino Acids* 40 (2011) 633-640.
- 336 [6] C. Torri, I. Lesci, D. Fabbri, Analytical study on the pyrolytic behaviour of cellulose in the presence of MCM-41  
 337 mesoporous materials, *J. Anal. Appl. Pyrolysis* 85 (2009) 2192-196.
- 338 [7] P. Rutkowski, Characteristics of bio-oil obtained by catalytic pyrolysis of beverage carton packaging waste, *J. Anal.*  
 339 *Appl. Pyrolysis* 98 (2012) 115-122.
- 340 [8] M. Staš, D. Kubička, J. Chudoba, M. Pospíšil, Overview of analytical methods used for chemical characterization of  
 341 pyrolysis bio-oil, *Energy Fuels* 28 (2014) 385-402.
- 342 [9] E. Alsbou, B. Helleur, Accelerated aging of bio-oil from fast pyrolysis of hardwood, *Energ. Fuel* 28 (2014) 3224-  
 343 3235.
- 344 [10] L. Zhang, C. Shen, R. Liu, GC-MS and FI-IR analysis of the bio-oil with addition of ethyl acetate during storage,  
 345 *Front. Energy Res.* (2014) 1-18.
- 346 [11] C. Lievens, D. Mourant, M. He, R. Gunawan, C. Z. Li, An FT-IR spectroscopic study of carbonyl functionalities in  
 347 bio-oils, *Fuel* 90 (2011) 3417-3423.
- 348 [12] P. K. Kanaujia, Y.K. Sharma, M.O. Garg, D. Tripathi, R. Singh, Review of analytical strategies in the production  
 349 and upgrading of bio-oils derived from lignocellulosic biomass, *J. Anal. Appl. Pyrolysis*, 10.1016/j.jaap.2013.10.004, in  
 350 press.
- 351 [13] L. Han, Q. Wang, Q. Ma, C. Yu, Z. Luo, K. Cen, Influence of CaO additives on wheat-straw pyrolysis as  
 352 determined by TG-FTIR analysis, *J. Anal. Appl. Pyrolysis* 88 (2013) 199-206.
- 353 [14] V. Castola, A. Bighelli, L. Conti, G. Scano, S. Mascia, J. Casanova, Identification and quantitation of  
 354 anhydrosugars in biomass pyrolytic oils using carbon-13 NMR spectroscopy, *Can. J. Anal. Sci. Spectrosc.* 45 (2000)  
 355 102-107.
- 356

- 357 [15] H. Ben, A. J. Ragauskas, Comparison for the compositions of fast and slow pyrolysis oils by NMR  
358 Characterization, *Bioresour. Technol.* 147 (2013) 577-584.
- 359 [16] M.M. Midland, K.E. Gilbert, Pcmol for windows, version 7.00, 1999. Serena Software; Bloomington, IN, USA.
- 360 [17] M. J. Frisch *et al.*, Gaussian 03, Revision C.02, 2004. Gaussian, Inc., Wallingford, CT.
- 361
- 362 [18] A. D Becke, Density-functional thermochemistry. iv. a new dynamical correlation functional and implications for  
363 exact-exchange mixing, *J. Chem.Phys.* 104 (1996) 1040-1046.
- 364 [19] Computational Chemistry Comparison and Benchmark DataBase. Precomputed vibrational scaling factors, 2013.  
365 <http://cccbdb.nist.gov/vibscale.asp>.
- 366 [20] M. Amutio, G. Lopez, M. Artetxe, G. Elordi, M. Olazar, J. Bilbao, Influence of temperature on biomass pyrolysis  
367 in a conical spouted bed reactor, *Resour. Conservat. Recycl.* 59 (2012) 23-31.
- 368 [21] H. F. Gercel, The effect of a sweeping gas flow rate on the fast pyrolysis of biomass, *Energy Sources* 24 (2002)  
369 633-643.
- 370 [22] S. K. Bharti, R. Roy, Quantitative  $^1\text{H}$ NMR spectroscopy, *Trends Anal. Chem.* 35 (2012) 5-26.
- 371 [23] J. P. Diebold, A Review of the Chemical and Physical Mechanisms of the Storage Stability of Fast Pyrolysis Bio-  
372 Oils, National Renewable Energy Laboratory Report, January 2000, NREL/SR-570-27613, Online available  
373 <http://gisceu.net/PDF/U918.pdf>.
- 374 [24] C. Lievens, D. Mourant, R. Gunawan, X. Li, C. Li, FT-IR carbonyl bands in bio-oils: Importance of water, *Fuel*  
375 112 (2013) 596-598.
- 376 [25] K. Smets, P. Adriaensen, J. Vandewijngaarden, M. Stals, T. Cornelissen, S. Schreurs, R. Carleer, J. Yperman,  
377 Water content of pyrolysis oil: Comparison between Karl Fischer titration, GC/MS-corrected azeotropic distillation and  
378  $^1\text{H}$  NMR spectroscopy, *J. Anal. Appl. Pyrolysis* 90 (2011) 100-105.
- 379
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## 381 Captions to figures

382 Fig. 1. Molecular structures of the main chiral anhydrosugars produced by a pyrolysis process.  
 383 Levoglucosan (LG), ascopyrone (AP), 1,6-anhydro- $\beta$ -D-glucofuranose (GFA), 2,3-anhydro-d-  
 384 mannosan (AM), dianhydro- $\beta$ -D-glucopyranose (DGP), (1R,5S)-1-hydroxy-3,6-dioxabicyclo[3.2.1]  
 385 octan-2-one (LAC) and levoglucosenone (LGO). Numbering on LAC structure are for convenience.

387 Fig. 2. Pyrolysis reactor consisting in a quartz phial (1) fixed on a stainless steel flange in which  
 388 two stainless steel pipes connect the reactor toward the flange, respectively to the nitrogen line (2)  
 389 and to the condensation apparatus (3). The sample is loaded through a third steel tube (4) into the  
 390 reaction volume by a porcelain combustion boat (5) and the temperature is provided by a tubular  
 391 furnace (6).

393 Fig. 3. NMR spectra of enriched bio-oil samples obtained by using catalysts Sn-MCM-41,  
 394 regenerated Sn-MCM-41, montmorillonite K10 (MK10) and aluminum-titanate (AlTi)  
 395 nanopowders, by cellulose pyrolysis at 500 °C. The two dotted lines highlight the LAC (d, 4.295  
 396 ppm, H-4ax) and LGO (d, 6.121 ppm, H-3) signals.

398 Fig. 4. Quantitative analysis by NMR standard-addition method. The curves of the four enriched  
 399 bio-oils were drawn by adding subsequent rates of LAC standard solution in  $\text{CDCl}_3$  and measuring  
 400 the peak area of the LAC signal (4.295 ppm) normalized on the LGO signal. The initial molar  
 401 concentration of LAC in each bio-oil solution in  $\text{CDCl}_3$  was obtained by extrapolation.

402 Fig. 5. Carbonyl region in FT-IR spectra for isopropanol solutions of pure LAC, levoglucosenone  
 403 (LGO), 5-(hydroxymethyl)-2-furaldehyde (HMF) and 2-furaldehyde (FF).

405 Fig. 6. Calibration curve for LAC quantification, obtained from FT-IR spectra of LAC solutions in  
 406 isopropanol at concentrations in the range 2÷104 mM (corresponding to 0.38÷15.00 mg mL<sup>-1</sup>).

408 Fig. 7. Carbonyl region in FT-IR spectra of the enriched bio-oils (25 mgmL<sup>-1</sup> isopropanol solutions)  
 409 obtained at 500 °C in different catalytic conditions: Sn-MCM-41, regenerated Sn-MCM-41,  
 410 montmorillonite K10 (MK10) and aluminium titanate (AlTi) nanopowders.

412 Fig. 8. FT-IR spectra of isopropanol solutions of pure LAC, enriched bio-oil obtained at 500 °C in  
 413 the presence of regenerated Sn-MCM-41 and water in different concentrations.

414 **Highlights**

415

- 416 - A selective production of LAC was upgraded by fast pyrolysis of cellulose.
- 417 - Pyrolysis at two temperatures, three catalysts and one regenerated was performed.
- 418 - LAC was quantified in bio-oils by a  $^1\text{H}$ -NMR standard-addition method.
- 419 - A fast quantitative IR evaluation of LAC in bio-oil solutions was also validated.

420

421

Table 1

LAC concentration in bio-oils produced by pyrolysis at 500 °C evaluated by quantitative  $^1\text{H}$ NMR method

Catalyst	LAC in bio-oil (wt.%)
Sn-MCM-41	$27.6 \pm 0.4$
Recycled Sn-MCM-41	$26.8 \pm 0.5$
MK10	$17.8 \pm 0.9$
AlTi	$10.9 \pm 0.5$

Table 2

LAC concentration in bio-oils produced by pyrolysis at the reported temperature evaluated by quantitative FT-IR method

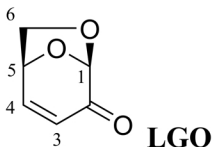
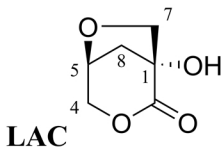
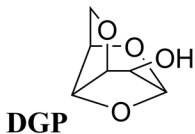
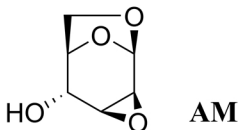
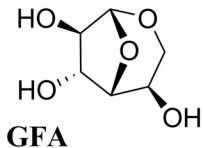
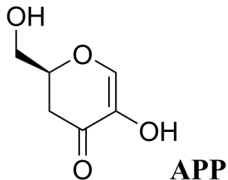
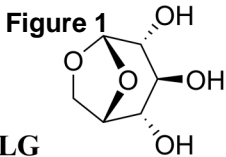
Catalyst	LAC in bio-oil (wt.%)	
	at 500°C	at 350°C
Sn-MCM-41	28.1±0.3	27.0±0.3
RecycledSn-MCM-41	27.3±0.3	25.4±0.3
MK10	18.6±0.2	24.1±0.3
AlTi	11.5±0.1	15.8±0.2

Table 3

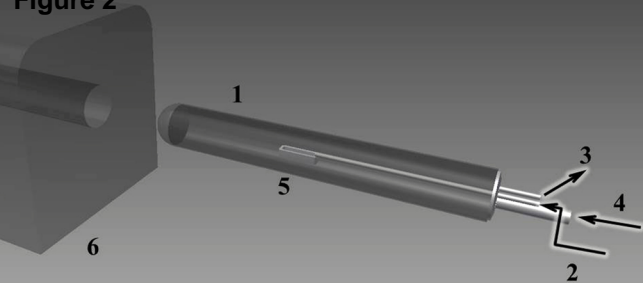
Comparative production of LAC by cellulose pyrolysis under different conditions, as established by quantitative FT-IR method

Catalyst	Yield of LAC (wt. %)	
	at 350°C	at 500°C
Sn-MCM-41	4.9	7.6
Recycled Sn-MCM-41	4.3	7.3
MK10	4.6	4.8
AlTi	2.4	2.6

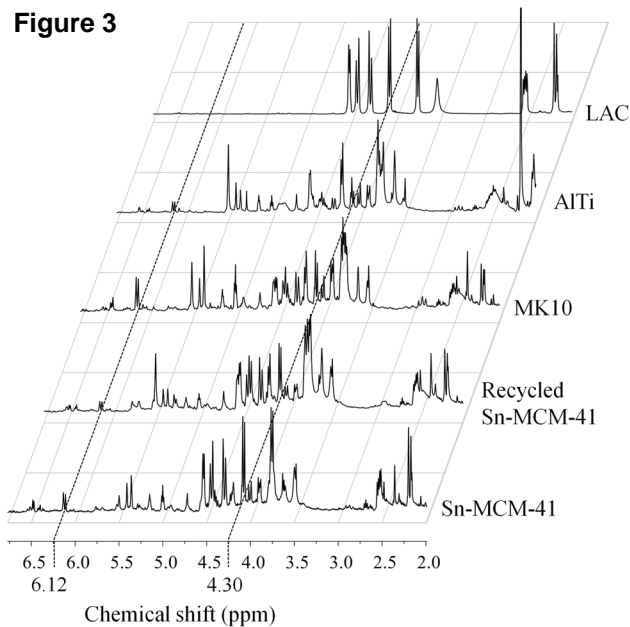




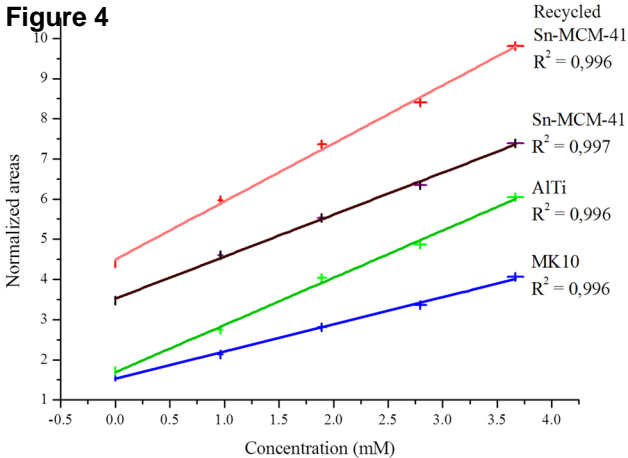
**Figure 2**



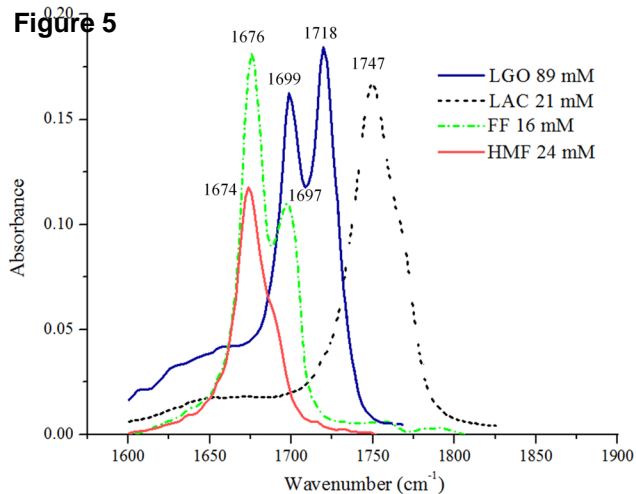
**Figure 3**



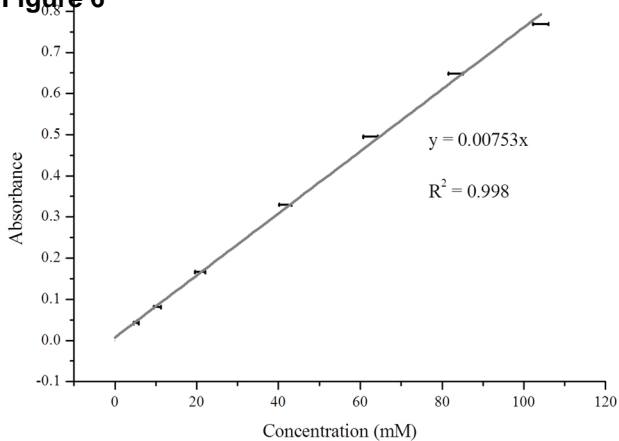
**Figure 4**



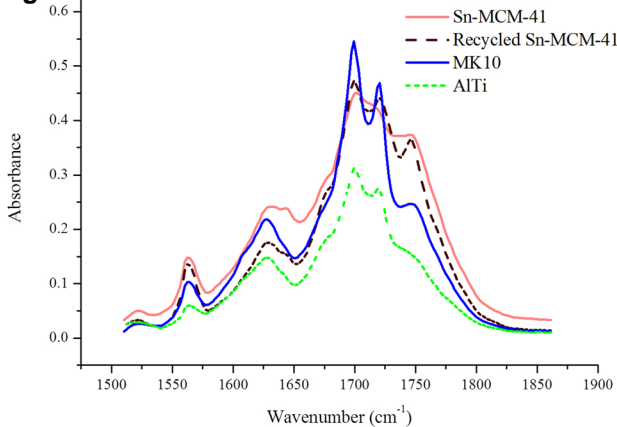
**Figure 5**



**Figure 6**



**Figure 7**



**Figure 8**

Bio-oil  
(recycled Sn-MCM-41)

--- LAC 42 mM

—  $\text{H}_2\text{O}$  0.56 M

—  $\text{H}_2\text{O}$  0.33 M

Absorbance

